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13. ABSTRACT (Maximum 200 Words)

During the progression of human PCa, hemidesmosomes are lost. Hemidesmosomes are adhesion structures that anchor epithelial cells to basement membrane and function as a tumor suppressor. We found that 12-lipoxygenase directly interacts with β4 integrin. We hypothesize that an increase in 12-LOX activity can cause the disassembly of hemidesmosomes, mobilization of α6β4 integrin from hemidesmosomes to other parts of the cell membrane, and stimulate tumor invasive growth. We proposed to conduct a correlation study using clinical tumor specimens. We will study whether 12(S)-HETE can disrupt hemidesmosomes and whether 12-LOX inhibitors promote the formation of hemidesmosomes. Then we will study the underlying signaling pathway, especially PKCα, initiated by 12(S)-HETE, in the disassembly of hemidesmosomes. Next we will overexpress β4 integrin and study the role of the interaction between 12-LOX and β4 integrin in the adhesion, proliferation, migration, and survival, in response to HGF/SF. Finally we will xenograft these transfected cells into mice, to evaluate whether any phenotypic changes of tumor cells in vitro can be recapitulated in vivo. The work will significantly advance our understanding about the complex process of prostate cancer progression as well as the possible role played by dietary fat in the progression of prostate cancer.

14. SUBJECT TERMS
Prostate cancer, hemidesmosome, 12-lipoxygenase, eicosanoid, HGF, SF

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INTRODUCTION

During the progression of human PCa, hemidesmosomes are lost. Hemidesmosomes are adhesion structures that anchor epithelial cells to basement membrane and function as a tumor suppressor. We found that 12-lipoxygenase directly interacts with β4 integrin. We hypothesize that an increase in 12-LOX activity can cause the disassembly of hemidesmosomes, mobilization of α6β4 integrin from hemidesmosomes to other parts of the cell membrane, and stimulate tumor invasive growth. We proposed to conduct a correlation study using clinical tumor specimens. We will study whether 12(S)-HETE can disrupt hemidesmosomes and whether 12-LOX inhibitors promote the formation of hemidesmosomes. Then we will study the underlying signaling pathway, especially PKCα, initiated by 12(S)-HETE, in the disassembly of hemidesmosomes. Next we will overexpress β4 integrin and study the role of the interaction between 12-LOX and β4 integrin in the adhesion, proliferation, migration, and survival, in response to HGF/SF. Finally we will xenograft these transfected cells into mice, to evaluate whether any phenotypic changes of tumor cells in vitro can be recapitulated in vivo. The work will significantly advance our understanding about the complex process of prostate cancer progression as well as the possible role played by dietary fat in the progression of prostate cancer.

BODY OF REPORT

List of Technical Objectives

1. Perform a correlation study in 100 cases of prostate cancer patients to evaluate whether loss of β4 polarized staining (dissolution of hemidesmosomes) is correlated with an increased 12-LOX expression and whether diffusive staining of β4 (mobilized β4) is co-localized with 12-LOX and correlated with tumor grade and stage.
2. Study the effects of 12-LOX inhibitors and 12(S)-HETE on hemidesmosome in prostate epithelium.
3. Study the signal transduction pathways that underlie the disassembly of hemidesmosomes by 12(S)-HETE or an increase in 12-LOX activity.
4. Overexpress β4 integrin in PC-3 cells, in the presence or absence of 12-LOX expression, and evaluate the capacity of transfected cells to form hemidesmosome and whether an increase in surface expression of α6β4 alters cell proliferation, adhesion, migration, and survival, in response to HGF/SF.
5. Evaluate the growth and progression of s.c. tumors derived from α6β4 expressing PC-3 cells, in the presence or absence of stable 12-LOX expression, and compare with that of control PC-3 cells.

PROGRESS

Task 1. Perform a correlation study in 100 cases of prostate cancer specimens to evaluate whether loss of β4 polarized staining (dissolution of hemidesmosomes) is correlated with an increased 12-LOX expression and whether diffusive staining of β4 (mobilized β4) is co-localized with 12-LOX and correlated with tumor grade and stage. Months 1 - 18:
This specific aim is still ongoing to optimize immunostaining for both 12-LOX and beta 4 integrin.

We have attempted several procedures for co-immunostaining for 12-LOX. We conducted immunohistochemical analysis of 12-LOX at the protein level in frozen human prostate tumor tissues (The antibody utilized does not work well with parafinn-embedded tissue, unpublished observations). As shown in the figure, 12-LOX immunoreactivity appears correlated with tumor grade. Neoplastic glands are weakly,

moderately or strongly positive for 12-LOX in low-grade tumor (**Figure 1A**), intermediate-grade tumor (**Figure 1 B, C**), or in high-grade tumor (**Figure 1 D**) correspondingly.

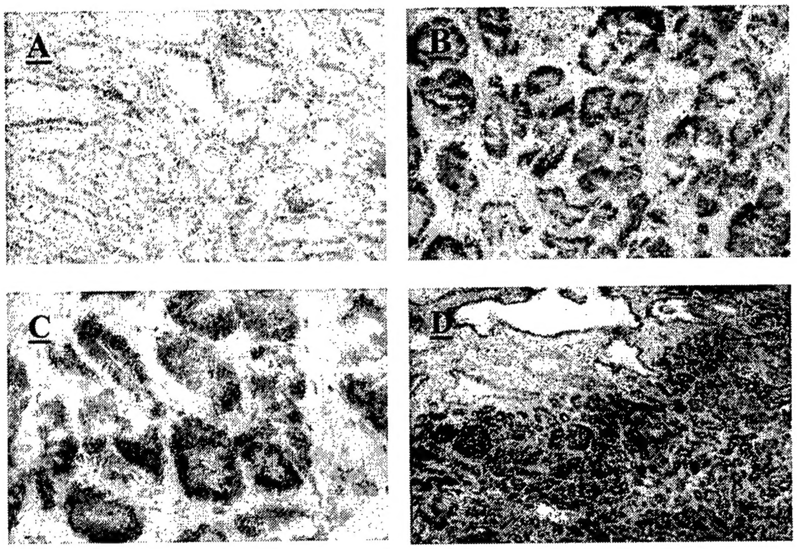

Figure 1. Immunohistochemical staining for 12-LOX in frozen prostate tumor tissues. Sections of frozen specimens were probed with a12-LOX polyclonal antibody (Oxford Biomedical Research Inc, Oxford, MI). Positive immunoreactivity is indicated by staining with brownish color. A, A low grade tumor; B & C, intermediate grade tumor; D, a high grade tumor.

We also attempted several protocols of immunostaining for beta 4 integrin. As shown in figure 2, focal positive staining was found in normal epithelium as well as in PIN.

A.

B.

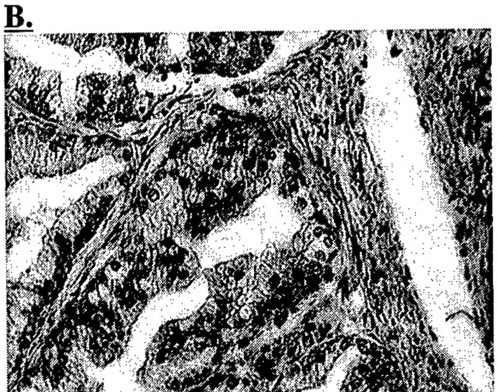

Figure 2. Immunostaining for beta 4 integrin. A. Normal prostate; B, PIN. Brownish color indicates positive staining. Note the pattern of beta 4 integrin positivity.

We have procured 100 cases of prostate tumor specimens. Once we have worked reliable protocol for double staining, the study will be completed in short order.

Task 2. Study the effects of 12-LOX inhibitors and 12(S)-HETE on hemidesmosome in prostate epithelium. Months 1-18:

This specific aim is partially achieved.

We studied the effect of inhibitors of 12-LOX as well as 12(S)-HETE on the hemidesmosome-like structures in cultured cells. As shown in Figure 3 and Figure 4, 12(S)-HETE (300 nM) reduced hemidesmosome like structures both in A431 cells and in an immortalized human prostate cancer cell line, CPTX1532. In contrast, 12-LOX inhibitor baicalein promoted the formation of hemidesmosome-like structure (Figure 4). Interestingly, a linoleic acid metabolite from 15-LOX, 13(S)-HODE, promoted the formation of hemidesmosomes (Figure 3).

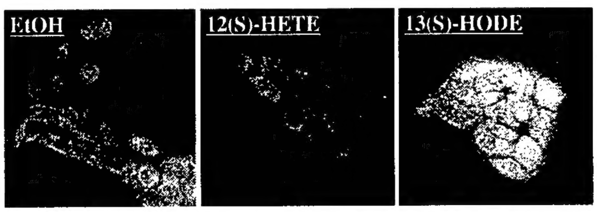

Figure 3: 12(S)-HETE reduced the focal staining of β4 integrin (middle) while 13(S)-HODE promoted the focal staining (right) in A431 cells. A431 cells were plated on matrigel and treated with 300 nM of 12(S)-HETE or 13(S)-HODE for 30 min, fixed with methanol/acetone, and then processed for immunostaining for β4 integrin. Note the marked increase in focal staining for β4 integrin in 13(S)-HODE treated group (right) vs. the control (EtOH, left). Also note the marked reduction in focal staining for β4 in 12(S)-HETE treated group (middle) when compared to the control (left).

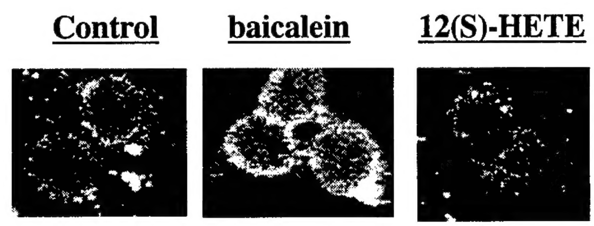

Figure 4: 12(S)-HETE reduced (right), while 12-LOX inhibitor baicalein promotes (middle), the polarized staining of β4 integrin in CPTX1532, a human prostate epithelial cell line immortalized with the E6 and E7 transforming proteins of human papilloma virus serotype 16. Cells were treated with baicailein or 12(S)-HETE for 30 min, fixed with methanol/acetone, and then processed for immunostaining for β4 integrin. Note the focal, or polarized staining pattern in baicalein treated group, and less in control group. Cells treated with 12(S)-HETE represented much less focal staining.

Task 3. Study the signal transduction pathways that underlie the disassembly of hemidesmosomes by 12(S)-HETE or an increase in 12-LOX activity, Months 12 -24:

The studies proposed have been initiated and are ongoing.

Task 4. Overexpress β4 integrin in PC-3 cells, in the presence or absence of 12-LOX expression, and evaluate the capacity of transfected cells to form hemidesmosomes and whether an increase in surface expression of α6β4 alters cell proliferation, adhesion, migration, and survival, in response to HGF/SF, Months 18 - 30:

The studies are in planning stage.

Task 5. Evaluate the growth rates of s.c. tumors derived from α6β4 expressing PC-3 cells, in the presence or absence of stable 12-LOX expression, and compare with that of control PC-3 cells, Months 24-36:

The studies proposed in this specific aim are in the stage of planning.

SUMMARY/Discussions:

Our studies found that 12-LOX physically interacts with the cytoplasmic tail of β4 integrin. The eicosanoid product of 12-LOX, 12(S)-HETE, dissolves hemidesmosomes while the inhibitors of 12-LOX such as baicalein and CDC enhance the formation of hemidesmosome-like structures. During the dissolution of hemidesmosome, β4 integrin is mobilized from hemidesmosome-like structure and distributes more diffusively in the cell membrane. Then 12-LOX interacts with the cytoplasmic tail of the mobilized or diffusive β4 and enhances PCa cell migration and invasion in response to HGF/SF. Further studies are needed to discern the direct effect of 12(S)-HETE and inhibitors of 12-LOX on hemidesmosome or hemidesmosome-like structures in normal prostate epithelia and whether 12(S)-HETE cause a mobilization of β4 integrin from hemidesmosome to diffusive distribution, and its role in tumor growth and progression.

KEY RESEARCH ACCOMPLISHMENT

2 research articles published
1 research article in submission
1 review article published

REPORTABLE OUTCOMES

- Research article published.
  Nie D, Nemeth J, Qiao Y, Zacharek A, Li L, Hanna K, Tang K, Hillman GG, Cher ML, Grignon DJ, Honn KV. Increased metastatic potential in human prostate carcinoma cells by overexpression of arachidonate 12-lipoxygenase. Clin Exp Metastasis. 2003;20(7):657-63.
- Research article published.
  Kandouz M, Nie D, Pidgeon GP, Krishnamoorthy S, Maddipati KR, Honn KV. Platelet-type 12-lipoxygenase activates NF-kappaB in prostate cancer cells. Prostaglandins Other Lipid Mediat. 2003 Jul;71(3-4):189-204.
- Research article published.
  Pidgeon GP, Tang K, Cai YL, Piasentin E and Honn KV. 2003. Overexpression of platelet type 12-lipoxygenase promotes tumor cell survival by enhancing aVbeta3 and aVbeta5 integrin expression. Cancer Res. 63: 4258-4267.
- Research article published.
  Pidgeon GP, Tang K, Rice RL, Zacharek A, Li L, Taylor JD, and Honn KV. 2003. Overexpression of leukocyte type 12-lipoxygenase promotes W256 tumor cell survival by enhancing aVbeta5 expression. Intl J Cancer 105: 459-471.
- Research article submitted.
  Nie, D., Y Chen, Y Qiao, A Zacharek, K Tang, J Milanini, G Pages, D Grignon, and KV Honn. Arachidonate 12-Lipoxygenase regulates the expression of vascular endothelial growth factor in a PI3 kinase dependent pathway.
- Review article published.
  Nie D, Honn KV.Eicosanoid regulation of angiogenesis in tumors. Semin Thromb Hemost. 2004 Feb;30(1):119-25.
- Review article published.
  Nie, D., M. Che, D. Grignon, K. Tang, and K. V. Honn. Role of eicosanoids in prostate cancer progression. Cancer and Metastasis Review 20: 195 – 206, 2001.
- Abstract published.

Krishnamoorthy, S., K. R. Maddipati, D. Nie, and K. V. Honn. 12-Lipoxygenase in hypoxia and hypoxia-induced angiogenesis. Proc. Amer. Assoc. Cancer Res. 45: #3591, 2004.

- Abstract published.

  Nie, D., Y. Qiao, A. Zacharek, and K. V. Honn. Blockade of NF-kB sensitizes prostate cancer cells to ionizing radiation. Proc. Amer. Assoc. Cancer Res. 45: #1290, 2004.

- Presentation.

  Nie, D, A. Zacharek, Q. Yan, and K. V. Honn. Differential Effects of Lipoxygenase Isoforms on Prostate Tumor Growth. Late Breaking Session of 94th Annual Meeting (2003) of American Association for Cancer Research. Washington, D.C., July, 2003.

- Presentation.

  Nie D., Yan Q., Zacharek A., Che M., and KV Honn. Inhibition of prostate tumor growth by 5-lipoxygenase and 15-lipoxygenase-2. 8th International Conference on Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation and Related Diseases. Chicago, IL. October, 2003.

- Presentation.

  Guo Y., **Nie D**., and KV Honn. Cloning and identification of a low affinity receptor for 12(S)-HETE. 8th International Conference on Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation and Related Diseases. Chicago, IL. October, 2003.

- Grant obtained. An R01 grant from NIH to study roles for eicosanoids in tumor metastasis.
- Development of animal models: No.